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December 2, 2009

Kimberly Whitlock
TechLaw, Inc.
205 W. Wacker Dr. Suite 1622
Chicago, IL 60606

**RE: Final Report: *Chironomus dilutus* and *Hyaella azteca* 10-Day Whole
Sediment Toxicity Testing Results For TechLaw Sediment Samples**

Dear Ms. Whitlock:

Great Lakes Environmental Center, Inc. (GLEC) has completed our analysis of the *Chironomus dilutus* (*tentans*) and *Hyaella azteca* 10-day whole sediment survival and growth toxicity tests performed with twelve sediment samples, collected by TechLaw personnel for whole sediment toxicity assessment. The sample identification numbers, survival, and growth test results for the twelve sediment samples are summarized and provided in the following tables:

- Table 1: 10-Day *C. dilutus* Average Percent Survival
- Table 2: 10-Day *C. dilutus* Biomass Estimates (expressed as average ash-free-dry-weight (AFDW))
- Table 3: 10-Day *H. azteca* Average Percent Survival
- Table 4: 10-Day *H. azteca* Biomass Estimates

Water quality data for the overlying water for each sediment sample tested are summarized in Table 5 for the *C. dilutus* tests and in Table 6 for the *H. azteca* tests. Daily overlying water quality measurements collected throughout the test are provided in Appendix B. Summaries of the statistical analyses conducted on the whole sediment toxicity test data are provided in Table 7 for the *C. dilutus* tests and in Table 8 for the *H. azteca* tests. Chain of Custody forms and reference toxicant data are provided in Appendices A and F, respectively.

(The daily laboratory bench data sheets are kept on file at GLEC and are also provided on the enclosed compact disks).

METHODS

The TechLaw sediment samples were analyzed at our Traverse City, Michigan laboratory following GLEC's written protocols which are based on the procedures outlined by: EPA/600/R-99/064 *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*, Second Edition; ASTM 1706-95B, *Standard Test Methods for Measuring the Toxicity of Sediment Associated Contaminants with Freshwater Invertebrates* (ASTM 2000); and GLEC Standard Operating Procedures (SOPs).

The twelve sediment samples were shipped by TechLaw personnel and received at GLEC, at which time they were assigned a unique GLEC laboratory identification number and stored at 4°C until test initiation (see table below).

Sample I.D.	GLEC Number	Date Sampled	Date Received
SPI-U16	7908	October 08, 2009	October 14, 2009
T29-25	7909	October 13, 2009	October 14, 2009
T26-50	7910	October 14, 2009	October 15, 2009
T25-50	7911	October 14, 2009	October 15, 2009
T17-50	7915	October 15, 2009	October 16, 2009
T24-75	7916	October 14, 2009	October 16, 2009
SPI-U15	7917	October 15, 2009	October 16, 2009
SPI-U10	7918	October 15, 2009	October 16, 2009
T23-75	7919	October 15, 2009	October 16, 2009
T22-25	7920	October 15, 2009	October 16, 2009
T20-175	7921	October 15, 2009	October 16, 2009
SPI-U04	7922	October 15, 2009	October 16, 2009

The 10-day *C. dilutus* and *H. azteca* toxicity tests were initiated on October 30, 2009, with each of the twelve investigative sediment samples and one laboratory control sediment with each test organism.

Second to third instar *C. dilutus* (10 days old at test initiation) and *H. azteca* (8-10 days old at test initiation) were used to initiate the whole sediment toxicity tests. *C. dilutus* and *H. azteca* were continuously exposed for 10 days to each of the sediment samples and to a laboratory control sediment. There were eight replicate beakers for each sediment sample and laboratory control; each replicate contained 10 animals. The laboratory control

sediment is a reference sediment collected locally from the Boardman River, a blue ribbon trout stream, located in the Pere Marquette State Forest.

The *C. dilutus* and *H. azteca* were exposed in 300 mL high form beakers, each containing 100 mL of whole sediment and 175 mL of overlying water. Overlying water was supplied to each test chamber at least twice daily (once every 12-hour period) via a static-renewal water delivery system. The overlying water consisted of de-chlorinated municipal (Lake Michigan) water of moderate hardness (160-180 mg/L). The *C. dilutus* test chambers were each fed 1.5 mL of Tetrafin® goldfish food slurry (4mg/mL dry solids) daily. The *H. azteca* test chambers were each fed 1.0 mL of YTC (1800 mg/L solids) daily.

The test chambers were placed in a temperature controlled water bath under the specified conditions of $23 \pm 1^\circ\text{C}$; photoperiod of 16 hours light and 8 hours dark; and ambient lighting. Water temperature was monitored continuously in the water bath using an electronic data logger (Appendix E) while temperatures in the test chambers were measured daily in two alternating replicates for each test sample. Alkalinity, hardness, pH, and total ammonia were measured at test initiation and at test termination (Tables 5 and 6). Observations on organism behavior and any anomalies observed within the sediment were made daily in each test chamber and recorded on the laboratory bench data sheets.

The number of *C. dilutus* surviving in each replicate test chamber was recorded at test termination (day 10); a summary of the percent survival is provided in Table 1.

The biomass, expressed as ash free dry weight [AFDW; in milligrams (mg)] of the surviving organisms divided by the initial number of organisms for each *C. dilutus* replicate, was determined at test termination and summarized in Table 2.

The number of surviving *H. azteca* in each test replicate chamber was recorded at test termination (day 10) and is summarized in Table 3. The biomass, expressed as the dry weight [(mg) of the surviving organisms divided by the initial number of organisms] for each *H. azteca* replicate, was determined at test termination and summarized in Table 4.

A statistical analysis using TOXSTAT (3.5, 1996) and statistical guidelines provided in EPA/600/R-99/064 *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*, Second Edition; and ASTM 1706-95B, *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (ASTM 2000), were used to compare the 10-day survival and growth endpoints. Initially, all survival data were transformed using an arc sine-square root transformation prior to analysis. The transformed data were then tested for normality and homogeneity of variances. Next, an analysis of variance (ANOVA) was conducted using the most appropriate parametric (e.g., Dunnet's or Bartlett's t-tests) or nonparametric (e.g., Steel's Many-One Rank or Wilcoxon

with Bonferroni's t-test. If the data failed to meet the assumptions of normality or homogeneity, the nonparametric tests were used to analyze the data. The investigative samples were considered statistically different when the percent survival was significantly lower ($p \leq 0.05$) than in the reference sediment. If survival in the investigative samples were significantly different ($p \leq 0.05$) from the reference sediment, growth was not used in the statistical comparisons. Those samples were considered significantly different from the reference sediment based solely on survival. If survival in the investigative samples were not significantly lower ($p \leq 0.05$) than in the reference sediment, growth was used in the statistical comparison.

Growth data were initially tested for normal distribution and homogeneity of variances. If the data were not normally distributed or homogenous then the data were transformed prior to conducting the most appropriate parametric or nonparametric tests. The growth was analyzed as biomass (average dry weight of surviving organisms divided by the number of initial organisms). The biomass (growth) for each investigative sample was considered statistically different when it was significantly lower ($p \leq 0.05$) than in the reference sediments.

GLEC laboratory controls for each toxicity test met the minimum survival and growth requirements as specified in the EPA /600/R-99/064 and those requirements are acknowledged in the following results section for each set of toxicity tests. In this instance, the laboratory control sediment survival and growth data were not used in statistical comparisons with the investigative sediment samples survival and growth data. The laboratory control sediment was used as a measure of test acceptability and health of the test organisms. All statistical analyses compared the investigative samples only to predetermined reference samples.

RESULTS

Chironomus dilutus

The *C. dilutus* exposed to the laboratory control sediment exceeded the minimum survival (70 percent or greater) and growth (0.48 mg or greater AFDW at test termination) criteria for an acceptable control (Tables 1 and 2). The acceptable requirements for survival and growth for the *C. dilutus* test can be found in EPA /600/R-99/064, Table 12.1. The overlying water quality measurements (Table 5) were also within the acceptable limits following the EPA testing protocol (i.e., daily mean temperatures were $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$; dissolved oxygen (DO) was maintained above 2.5 mg/L in the overlying water; and there were no variations greater than 50% in overlying water hardness, alkalinity, or total ammonia measurements within each test type). Consequently, the *C. dilutus* whole

sediment toxicity tests were conducted following the standard protocols and are valid assessments of sediment toxicity.

The following exceptions were noted during the toxicity tests. On November 3, 2009, the DO in the overlying water of investigative sediment sample SPI-U-4, fell below 2.5 mg/L. In response, additional overlying water renewals were added to all of the controls and investigative test sediments. The brief drop in DO was unlikely to have affected the test results (see EPA /600/R-99/064 manual, section 12.3.6.2.2). All test chambers were observed daily to assess organism behavior and no unusual observations were noted with the test organisms.

Reference Sample SPI-U16 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *C. dilutus* survival for two of the eight investigative samples (T29-25 and T25-50) after 10 days of exposure, when compared to reference sample SPI-U16 sediment (Tables 1, 2, and 7). The *C. dilutus* growth expressed as biomass was significantly reduced ($p \leq 0.05$) in investigative sediment samples; T26-50, T17-50, T24-75, T23-75, T22-25, and T20-175, when compared to the reference sample SPI-U16 sediment (Tables 2 and 7).

Reference Sample SPI-U15 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *C. dilutus* survival for one of the eight investigative samples (T29-25) after 10 days of exposure, when compared to reference sample SPI-U15 sediment (Tables 1, 2, and 7). *C. dilutus* growth expressed as biomass was not significantly reduced ($p \geq 0.05$) in the seven investigative sediment samples when compared to the reference sample SPI-U15 sediment (Tables 2 and 7).

Reference Sample SPI-U10 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *C. dilutus* survival for five of the eight investigative samples (T29-25, T26-50, T25-50, T17-50, and T22-25) after 10 days of exposure, when compared to reference sample SPI-U10 sediment (Tables 1, 2, and 7). The *C. dilutus* growth expressed as biomass was significantly reduced ($p \leq 0.05$) in the investigative sediment samples T24-75 and T23-75 when compared to the reference sample SPI-U10 sediment (Tables 2 and 7).

Reference Sample SPI-U04 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *C. dilutus* survival for four of the eight investigative samples (T29-25, T26-50, T25-50, and T22-25) after 10 days of

exposure, when compared to reference sample SPI-U04 sediment (Tables 1, 2, and 7). The *C. dilutus* growth expressed as biomass was significantly reduced ($p \leq 0.05$) in the three investigative sediment samples T24-75, T23-75, and T17-50, when compared to the reference sample SPI-U04 sediment (Tables 2 and 7).

A summary of the survival and growth statistical analysis for the *C. dilutus* whole sediment toxicity tests is provided in Tables 1, 2, and 7, and Appendix C.

Hyaella azteca

The *H. azteca* exposed to the laboratory control sediment exceeded the minimum survival criterion (80%) and had measurable growth relative to the weight of organisms at test initiation (Tables 3 and 4). The requirements for acceptable survival and growth for the *H. azteca* can be found in EPA /600/R-99/064, Table 11.2. The overlying water quality measurements (Table 6) were also within the acceptable limits following the EPA testing protocol (i.e., daily mean temperatures were 23 ± 1 °C; DO was maintained above 2.5 mg/L in the overlying water; and there were no variations greater than 50% in overlying water hardness, alkalinity, or total ammonia measurements within each test type). All test chambers were checked daily to assess organism behavior and no unusual observations were noted. Consequently, the *H. azteca* whole sediment toxicity tests are valid assessments of sediment toxicity.

The following exceptions were noted during the toxicity tests. On November 4, 2009, the DO in the overlying water of two investigative sediment samples SPI-U16 and SPI-U04, fell below 2.5 mg/L. In response, additional overlying water renewals were added to all of the controls and investigative test sediments. The brief drop in DO was unlikely to have affected the test results (see EPA /600/R-99/064 manual).

Reference Sample SPI-U16 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *H. azteca* survival for seven of the eight investigative samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, and T22-25) after 10 days of exposure, when compared to reference sample SPI-U16 sediment (Tables 3, 4, and 8). The *H. azteca* growth expressed as biomass was significantly reduced ($p \leq 0.05$) in the investigative sediment sample T20-175 when compared to the reference sample SPI-U16 sediment (Tables 4 and 8).

Reference Sample SPI-U15 Compared to Investigative Sediment Samples

Reference sample SPI-U15 had zero percent survival after 10 days of exposure. Therefore, statistical comparisons for percent survival and biomass were not analyzed.

Reference Sample SPI-U10 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *H. azteca* survival for seven of the eight investigative samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, and T22-25) after 10 days of exposure, when compared to reference sample SPI-U10 sediment (Tables 3, 4, and 8). The *H. azteca* growth expressed as biomass was not significantly reduced ($p \leq 0.05$) in the investigative sediment sample T20-175 when compared to the reference sample SPI-U10 sediment (Tables 4 and 8).

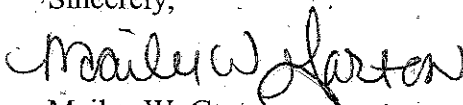
Reference Sample SPI-U04 Compared to Investigative Sediment Samples

There was a statistically significant reduction ($p \leq 0.05$) in *H. azteca* survival for seven of the eight investigative samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, and T22-25) after 10 days of exposure, when compared to reference sample SPI-U04 sediment (Tables 3, 4, and 8). The *H. azteca* growth expressed as biomass was significantly reduced ($p \leq 0.05$) in the investigative sediment sample T17-50 when compared to the reference sample SPI-U04 sediment (Tables 4 and 8).

A summary of the survival and growth statistical analysis for the *H. azteca* whole sediment toxicity tests is provided in Tables 3, 4, and 8, and Appendix D.

If you have any questions regarding the results of these sediment toxicity tests, or if you would like additional information, please contact either me or Dennis McCauley at (231) 941-2230. Thank you for the opportunity to provide this service to TechLaw Inc. and we look forward to continuing to provide environmental services to you in the future.

Sincerely,



Mailee W. Garton
Laboratory Coordinator

MWG:mg



TABLE 1. Comparison of Number of Surviving Organisms per Replicate and Percent Survival Between the Reference Sediment Samples (SPI-U16, SPI-U15, SPI-U10, and SPI-U04) and Investigative Sediment Samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, T22-25, and T20-175) for the TechLaw *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009

REPLICATE NUMBER	Number Test Organisms Surviving per Replicate ^r												
	Laboratory Control	SPI-U16 GLC# 7908	SPI-U15 GLC# 7917	SPI-U10 GLC# 7918	SPI-U04 GLC# 7922	T29-25 GLC# 7909	T26-50 GLC# 7910	T25-50 GLC# 7911	T17-50 GLC# 7915	T24-75 GLC# 7916	T23-75 GLC# 7919	T22-25 GLC# 7920	T20-175 GLC# 7921
1	9	9	4	10	9	1	10	8	10	9	9	9	10
2	7	8	8	10	10	0	8	5	8	10	10	7	10
3	9	10	5	10	10	0	8	8	9	10	9	8	10
4	10	10	7	10	10	4	5	8	9	10	9	9	10
5	10	9	5	10	10	0	5	6	10	10	10	9	9
6	10	10	4	10	10	1	8	7	6	9	8	10	10
7	9	10	0	10	10	3	7	8	9	10	10	9	10
8	10	10	5	10	10	0	10	7	9	10	7	9	9
10-Day Percent Survival	92.5	95.0	47.5	100.0	98.8	a, b,c,d 11.3	c,d 76.3	a,c,d 71.3	c 87.5	97.5	90.0	c,d 87.5	97.5

^a Significantly different ($p \leq 0.05$) from reference sediment SPI-U16 (GLC# 7908)

^b Significantly different ($p \leq 0.05$) from reference sediment SPI-U15 (GLC# 7917)

^c Significantly different ($p \leq 0.05$) from reference sediment SPI-U10 (GLC# 7918)

^d Significantly different ($p \leq 0.05$) from reference sediment SPI-U04 (GLC# 7922)

^r Replicates initiated with 10 organisms each



TABLE 2. Comparison of Biomass¹ Ash-Free-Dry Weight (AFDW) (mg) and Percent Survival Between the Reference Sediment Samples (SPI-U16, SPI-U15, SPI-U10, and SPI-U04) and Investigative Sediment Samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, T22-25, and T20-175) for the TechLaw *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009

REPLICATE #	Laboratory Control	SPI-U16 GLC# 7908	SPI-U15 GLC# 7917	SPI-U10 GLC# 7918	SPI-U04 GLC# 7922	T29-25 GLC# 7909	T26-50 GLC# 7910	T25-50 GLC# 7911	T17-50 GLC# 7915	T24-75 GLC# 7916	T23-75 GLC# 7919	T22-25 GLC# 7920	T20-175 GLC# 7921
	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)
1	1.39	1.24	0.01	0.86	0.95	0.07	0.22	0.13	0.19	0.40	0.37	0.56	1.03
2	0.93	1.03	0.11	0.92	0.77	0.00	0.14	0.07	0.22	0.46	0.48	0.33	0.77
3	1.14	1.32	0.08	0.88	1.02	0.00	0.13	0.13	0.26	0.51	0.31	0.45	0.92
4	1.21	1.20	0.11	0.92	0.75	0.09	0.15	0.14	0.16	0.69	0.45	0.25	1.00
5	1.29	1.06	0.11	0.78	0.88	0.00	0.13	0.14	0.23	0.44	0.44	0.58	0.92
6	1.05	1.21	0.06	0.82	0.79	0.01	0.26	0.11	0.13	0.27	0.30	0.41	0.70
7	1.51	1.22	0.00	1.04	0.95	0.04	0.14	0.10	0.18	0.43	0.33	0.43	0.91
8	1.87	1.59	0.10	0.94	0.79	0.00	0.21	0.09	0.23	0.45	0.30	0.40	1.06
Average Biomass ² Weight (AFDW) (mg)	1.30	1.23	0.07	0.89	0.86	e 0.03	a, e 0.17	e 0.11	a, d, e 0.20	a,c,d 0.45	a, c, d 0.37	a, e 0.42	a 0.91
10-Day Percent Survival	92.5	95.0	47.5	100.0	98.8	a, b,c, d 11.3	c, d 76.3	a, c, d 71.3	c 87.5	97.5	90.0	c, d 87.5	97.5

¹ Biomass weight is the total ash-free-dry weight of surviving organisms divided by the initial number of organisms

^a Significantly different ($p \leq 0.05$) from reference sediment SPI-U16 (GLC# 7908)

^b Significantly different ($p \leq 0.05$) from reference sediment SPI-U15 (GLC# 7917)

^c Significantly different ($p \leq 0.05$) from reference sediment SPI-U10 (GLC# 7918)

^d Significantly different ($p \leq 0.05$) from reference sediment SPI-U04 (GLC# 7922)

^e Survival in investigative samples which were significantly different ($p \leq 0.05$) from the reference sediments were not used in the growth comparisons.

Note: Average Ash-Free-Dry Weight AFDW) of *Chironomus dilutus* at test initiation = 0.354 mg



TABLE 3. Comparison of Number of Surviving Organisms per Replicate and Percent Survival Between the Reference Sediment Samples (SPI-U16, SPI-U15, SPI-U10, and SPI-U04) and Investigative Sediment Samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, T22-25, and T20-175) for the TechLaw *Hyaella azteca* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009

REPLICATE NUMBER	Number Test Organisms Surviving per Replicate ^r												
	Laboratory Control	SPI-U16 GLC# 7908	SPI-U15 GLC# 7917	SPI-U10 GLC# 7918	SPI-U04 GLC# 7922	T29-25 GLC# 7909	T26-50 GLC# 7910	T25-50 GLC# 7911	T17-50 GLC# 7915	T24-75 GLC# 7916	T23-75 GLC# 7919	T22-25 GLC# 7920	T20-175 GLC# 7921
1	10	8	0	10	10	0	4	6	8	4	0	0	4
2	9	8	0	8	10	0	5	6	6	0	4	2	7
3	9	9	0	10	9	0	3	1	7	4	2	0	9
4	8	10	0	8	9	0	7	1	5	4	2	0	9
5	10	8	0	7	8	0	5	1	1	5	1	0	9
6	9	6	0	8	10	0	2	1	3	4	0	0	8
7	10	9	0	8	9	0	2	0	2	1	0	2	10
8	10	9	0	6	9	0	1	1	4	1	1	0	8
10-Day Percent Survival	93.8	83.8	0.0[*]	81.3	92.5	a, c, d 0.0	a, c, d 36.3	a, c, d 21.3	a, c, d 45.0	a, c, d 28.8	a, c, d 12.5	a, c, d 5.0	80.0

^a Significantly different ($p \leq 0.05$) from reference sediment SPI-U16 (GLC# 7908)

^c Significantly different ($p \leq 0.05$) from reference sediment SPI-U10 (GLC# 7918)

^d Significantly different ($p \leq 0.05$) from reference sediment SPI-U04 (GLC# 7922)

^{*} Reference sediment SPI-U15 was not compared statistically to investigative sediment samples due to 100 percent mortality.

^r Replicates initiated with 10 organisms each



TABLE 4. Comparison of Biomass¹ Dry Weight (mg) and Percent Survival Between the Reference Sediment Samples (SPI-U16, SPI-U15, SPI-U10, and SPI-U04) and Investigative Sediment Samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, T22-25, and T20-175) for the TechLaw *Hyaella azteca* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009

REPLICATE #	Laboratory Control	SPI-U16 GLC# 7908	SPI-U15 GLC# 7917	SPI-U10 GLC# 7918	SPI-U04 GLC# 7922	T29-25 GLC# 7909	T26-50 GLC# 7910	T25-50 GLC# 7911	T17-50 GLC# 7915	T24-75 GLC# 7916	T23-75 GLC# 7919	T22-25 GLC# 7920	T20-175 GLC# 7921
	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)	Biomass ² Weight (mg)
1	0.09	0.08	0.00	0.04	0.07	0.00	0.02	0.02	0.03	0.01	0.00	0.00	0.01
2	0.08	0.08	0.00	0.06	0.07	0.00	0.02	0.03	0.02	0.00	0.01	0.01	0.04
3	0.07	0.07	0.00	0.04	0.07	0.00	0.01	0.01	0.04	0.01	0.01	0.00	0.04
4	0.05	0.06	0.00	0.04	0.06	0.00	0.02	0.00	0.02	0.03	0.01	0.00	0.03
5	T.E.	0.03	0.00	0.04	0.05	0.00	0.02	0.01	0.02	0.03	0.01	0.00	0.04
6	0.08	0.04	0.00	0.04	0.05	0.00	0.01	0.02	0.02	0.02	0.00	0.00	0.03
7	0.10	0.04	0.00	0.04	0.10	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.04
8	0.09	0.04	0.00	0.03	0.05	0.00	0.00	0.01	0.03	0.02	0.01	0.00	0.03
Average Biomass ² Weight (mg)	0.08	0.05	0.00 [*]	0.04	0.06	0.00 ^e	0.01 ^e	0.01 ^e	0.02 ^e	0.02 ^e	0.01 ^e	0.00 ^e	0.03 ^{a, d}
10-Day Percent Survival	93.8	83.8	0.0 [*]	81.3	92.5	a, c, d 0.0	a, c, d 36.3	a, c, d 21.3	a, c, d 45.0	a, c, d 28.8	a, c, d 12.5	a, c, d 5.0	

¹ Biomass weight is the total dry weight of surviving organisms divided by the initial number of organisms

^a Significantly different ($p \leq 0.05$) from reference sediment SPI-U16 (GLC# 7908)

^c Significantly different ($p \leq 0.05$) from reference sediment SPI-U10 (GLC# 7918)

^d Significantly different ($p \leq 0.05$) from reference sediment SPI-U04 (GLC# 7922)

^e Survival in investigative samples which were significantly different ($p \leq 0.05$) from the reference sediments were not used in the growth comparisons.

*Reference sediment SPI-U15 was not compared statistically to investigative sediment samples due to 100 percent mortality.

Note: Average Dry Weight of *Hyaella azteca* at test initiation = 0.02 mg

T.E.: Technician Error

TABLE 5. Summary of Mean Water Quality Parameters of Overlying Water Samples Collected Prior to Renewal for the TechLaw *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009.

Sample ID GLC #	Temperature °C <i>n</i> =22	pH (s.u.) <i>n</i> =3	Dissolved Oxygen (mg/L) <i>n</i> =22	Specific Conductivity (μmhos) <i>n</i> =4	Alkalinity (mg/L CaCO ₃) <i>n</i> =2	Hardness (mg/L CaCO ₃) <i>n</i> =2	Total Ammonia (mg/L) <i>n</i> =2
Laboratory Control	22.7 (22.2-23.8)	7.67	4.2 (2.4-6.3)	301	102	136	0.79
SPI-U16 GLC# 7908	22.7 (22.0-23.8)	7.80	4.2 (2.0-6.4)	302	108	146	0.32
T29-25 GLC# 7909	22.7 (22.1-23.7)	8.32	5.1 (3.8-5.9)	434	146	118	1.16
T26-50 GLC# 7910	22.7 (22.1-23.7)	8.03	4.5 (2.2-6.9)	376	147	142	4.43
T25-50 GLC# 7911	22.7 (22.0-23.6)	8.12	4.9 (2.7-6.5)	362	145	142	6.17
T17-50 GLC# 7915	22.7 (22.0-23.6)	7.91	5.0 (3.5-6.4)	296	105	136	0.43
T24-75 GLC# 7916	22.7 (22.0-23.7)	8.34	5.2 (3.5-6.5)	304	93	122	0.84
SPI-U15 GLC# 7917	22.7 (22.0-23.8)	8.02	5.0 (3.2-6.6)	309	117	144	0.59
SPI-U10 GLC# 7918	22.7 (22.0-23.7)	8.02	5.0 (2.5-6.8)	302	110	142	0.46
T23-75 GLC# 7919	22.7 (22.0-23.8)	8.49	5.3 (3.6-7.3)	256	84	368	0.47
T22-25 GLC# 7920	22.7 (22.0-23.8)	8.86	5.4 (3.5-6.3)	469	182	90	0.64
T20-175 GLC# 7921	22.7 (22.1-23.7)	8.38	5.4 (3.5-6.9)	369	91	138	0.19
SPI-U04 GLC# 7922	22.6 (22.1-23.6)	7.63	3.2 (1.3-5.9)	349	93	144	4.05

TABLE 6. Summary of Mean Water Quality Parameters of Overlying Water Samples Collected Prior to Renewal for the TechLaw *Hyaella azteca* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009.

Sample ID GLC #	Temperature °C <i>n</i> =22	pH (s.u.) <i>n</i> =3	Dissolved Oxygen (mg/L) <i>n</i> =22	Specific Conductivity (μmhos) <i>n</i> =4	Alkalinity (mg/L CaCO ₃) <i>n</i> =2	Hardness (mg/L CaCO ₃) <i>n</i> =2	Total Ammonia (mg/L) <i>n</i> =2
Laboratory Control	22.6 (22.0-23.1)	7.78	5.3 (1.7-6.5)	301	101	134	0.67
SPI-U16 GLC# 7908	22.6 (22.1-22.9)	7.93	5.7 (2.3-6.7)	302	108	142	0.26
T29-25 GLC# 7909	22.6 (22.0-22.9)	8.37	5.8 (4.5-7.0)	430	143	116	0.89
T26-50 GLC# 7910	22.6 (22.0-23.0)	8.09	5.4 (4.0-6.9)	375	146	142	4.18
T25-50 GLC# 7911	22.7 (22.1-23.1)	8.16	5.6 (3.7-7.0)	360	143	140	5.86
T17-50 GLC# 7915	22.7 (22.2-23.0)	7.98	5.7 (4.5-7.1)	295	104	132	0.31
T24-75 GLC# 7916	22.6 (22.0-22.9)	8.42	6.1 (4.2-7.0)	301	92	120	0.70
SPI-U15 GLC# 7917	22.6 (22.1-22.9)	8.09	5.6 (4.2-6.6)	309	115	144	0.53
SPI-U10 GLC# 7918	22.6 (22.0-23.0)	8.05	6.2 (3.2-7.2)	302	109	134	0.41
T23-75 GLC# 7919	22.6 (22.1-23.0)	8.59	6.2 (4.2-7.3)	254	83	370	0.34
T22-25 GLC# 7920	22.7 (22.0-23.1)	9.00	6.2 (4.4-7.2)	466	181	92	0.51
T20-175 GLC# 7921	22.6 (22.0-23.1)	8.43	6.6 (4.0-7.5)	365	88	136	0.06
SPI-U04 GLC# 7922	22.5 (22.1-23.1)	7.60	4.1 (1.5-5.9)	345	89	144	3.75

TABLE 7. Summary of Statistically Significant Differences ($p \leq 0.05$) Between the Investigative Sediment Samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, T22-25, and T20-175) and the Reference Sediment Samples (SPI-U16, SPI-U15, SPI-U10, and SPI-U04) for the TechLaw *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009.

Test Material	GLC #	10-Day Survival with SPI-U16	10-Day Biomass with SPI-U16	10-Day Survival with SPI-U15	10-Day Biomass with SPI-U15	10-Day Survival with SPI-U10	10-Day Biomass with SPI-U10	10-Day Survival with SPI-U04	10-Day Biomass with SPI-U04
T29-25	7909	A	*	B	*	C	*	D	*
T26-50	7910		A			C	*	D	*
T25-50	7911	A	*			C	*	D	*
T17-50	7915		A			C	*		D
T24-75	7916		A				C		D
T23-75	7919		A				C		D
T22-25	7920		A			C	*	D	*
T20-175	7921		A						

A-Significantly different ($p \leq 0.05$) from reference sediment SPI-U16 (GLC# 7908)

B-Significantly different ($p \leq 0.05$) from reference sediment SPI-U15 (GLC# 7917)

C-Significantly different ($p \leq 0.05$) from reference sediment SPI-U10 (GLC# 7918)

D-Significantly different ($p \leq 0.05$) from reference sediment SPI-U04 (GLC# 7922)

*- Survival in investigative samples which were significantly different ($P = 0.05$) from the reference sediments were not used in the growth comparisons.

Average Growth: Total Ash-Free-Dry Weight divided by number surviving organisms.

Biomass Growth: Total Ash-Free-Dry Weight divided by number of initial organisms.



TABLE 8. Summary of Statistically Significant Differences ($p \leq 0.05$) Between the Investigative Sediment Samples (T29-25, T26-50, T25-50, T17-50, T24-75, T23-75, T22-25, and T20-175) and the Reference Sediment Samples (SPI-U16, SPI-U15, SPI-U10, and SPI-U04) for the TechLaw *Hyaella azteca* 10-Day Whole Sediment Toxicity Tests Conducted October 30-November 09, 2009.


Test Material	GLC #	10-Day Survival with SPI-U16	10-Day Biomass with SPI-U16	10-Day Survival with SPI-U15	10-Day Biomass with SPI-U15	10-Day Survival with SPI-U10	10-Day Biomass with SPI-U10	10-Day Survival with SPI-U04	10-Day Biomass with SPI-U04
T29-25	7909	A	*			C	*	D	*
T26-50	7910	A	*			C	*	D	*
T25-50	7911	A	*			C	*	D	*
T17-50	7915	A	*			C	*	D	*
T24-75	7916	A	*			C	*	D	*
T23-75	7919	A	*			C	*	D	*
T22-25	7920	A	*			C	*	D	*
T20-175	7921		A						D

A-Significantly different ($p \leq 0.05$) from reference sediment SPI-U16 (GLC# 7908)

C-Significantly different ($p \leq 0.05$) from reference sediment SPI-U10 (GLC# 7918)

D-Significantly different ($p \leq 0.05$) from reference sediment SPI-U04 (GLC# 7922)

*- Survival in investigative samples which were significantly different ($P = 0.05$) from the reference sediments and were not used in the growth comparisons.

 Reference sediment SPI-U15 was not compared statistically to investigative sediment samples due to 100 percent mortality.

Biomass Growth: Total Dry Weight divided by number of initial organisms.